Water Qual. Res. J. Canada, 2004 • Volume 39, No. 3, 160–174

Development and Survivorship of Northern Leopard Frogs (*Rana pipiens*) and Green Frogs (*Rana clamitans*) Exposed to Contaminants in the Water and Sediments of the St. Lawrence River near Cornwall, Ontario

Tana V. McDaniel,[1] Megan L. Harris,[2] Christine A. Bishop[3] and John Struger[4]*

[1]*Canadian Wildlife Service, Environment Canada, 867 Lakeshore Road, P.O. Box 5050, Burlington, Ontario L7R 4A6*
[2]*Lorax Environmental, 19 Te Toroa Drive, RD5 Lake Tarawera, Rotorua, New Zealand*
[3]*Pacific Wildlife Research Centre, 5421 Robertson Road, R.R. 1, Delta, British Columbia V4K 3N2*
[4]*Ecosystem Health Division, Environmental Conservation Branch, Environment Canada, 867 Lakeshore Road, P.O. Box 5050, Burlington, Ontario L7R 4A6*

High levels of contamination in the aquatic environment and wildlife within the Ontario portion of the St. Lawrence River at the Cornwall Area of Concern (AOC) have raised questions about potential impacts on wildlife health. Northern leopard frog embryos were raised in two wetland sites within the AOC and at two reference sites to assess differences in water and sediment quality on survivorship and deformity rates. Chlorinated hydrocarbons (total polychlorinated biphenyls, organochlorine pesticides), polycyclic aromatic hydrocarbons, nutrients and heavy metals were measured in sediment and/or water from the study sites. Levels of some metals such as aluminium, cadmium, chromium and copper, exceeded federal and provincial guidelines for the protection of aquatic life, especially in the two AOC wetlands. Early stage tadpole survivorship was significantly lower and deformity frequency significantly higher at wetlands within the AOC; however, differences were likely not biologically significant. Survivorship and deformity rates of leopard frogs (*Rana pipiens*) at metamorphosis did not differ significantly among sites. Onset of metamorphosis was accelerated in tadpoles raised in wetlands within the AOC. Tadpoles raised in wetlands within the St. Lawrence River AOC took significantly less time to complete metamorphosis (53–55 days) than did tadpoles raised at reference sites (61–64 days). The phenology of metamorphosis was also more synchronous in tadpoles raised in the AOC, with all tadpoles reaching metamorphosis within a space of 3 to 7 days, as compared to 9 to 12 days at reference wetlands; these differences could not be accounted for by water temperature. Differences in development and survivorship rates between AOC and reference sites may be related to contaminant concentrations in water and sediment. However, no strong evidence for beneficial use impairment in terms of reproductive impairments or elevated deformity rates were seen from caged leopard frogs in the two AOC wetlands.

Key words: **amphibians, deformities, St. Lawrence River, Cornwall Area of Concern**

Introduction

Lake St. Francis and the upstream portion of the St. Lawrence River where it passes through Cornwall (Ontario) and Massena (New York) have been designated by the International Joint Commission (International Joint Commission 1978) as an Area of Concern (AOC), due to high levels of contamination in the sediments from present day and historic industrial activity. Elevated contamination levels in the AOC have been associated with high tissue contaminant burdens and potential health effects in local wildlife. Elevated levels of PCBs, as high as 737 µg/g were measured in snapping turtle (*Chelydra serpentina*) eggs collected from sites along the south shore of the Cornwall AOC; this represents one of the highest tissue concentrations ever recorded in wildlife (de Solla et al. 2001). Tree swallows (*Tachycineta bicolor*) from some sites within the AOC exhibited a reduction in basal plasma corticosterone levels associated with elevated levels of PCDFs in the environment (Martinovic et al. 2003).

The south shore of the river is characterized by high levels of polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) in the sediments, while the north shore is characterized by high levels of mercury and heavy metals in the sediments (Dreier et al. 1997). The International Joint Commission (International Joint Commission 1987) has defined 14 ways that beneficial uses of water may be impaired in the Great Lakes, many of which are considered impaired in the St. Lawrence (at Cornwall) AOC. These include: degradation of the benthos, restrictions on dredging due to

* Corresponding author; john.struger@ec.gc.ca

high contaminant loads in the sediments, excess eutrophication, degradation of fish and wildlife populations, restrictions of fish and wildlife consumption due to high mercury and PCB body burdens and degradation of fish and wildlife habitat (Dreier et al. 1997).

Beneficial use impairment in the form of deformities or reproductive problems in wildlife is still under investigation and should be determined as part of the remedial action plan for the Cornwall AOC. There is some evidence to suggest that amphibian deformities may be elevated within the AOC. Mudpuppies *(Necturus maculosus)* from the St. Lawrence as it passes through the Cornwall AOC exhibited increased rates of skeletal deformities (Bishop and Gendron 1998). Unpublished surveys of leopard frogs on the north shore of the AOC also indicated potentially elevated incidences of deformities (B. Hickey, pers. comm.). Elevated rates of deformity in amphibians have been associated with heavy metal and PCB contamination in studies from other localities (Calevro et al. 1998). Although little field work has been conducted, laboratory exposures suggest that contaminant levels in the AOC represent a threat to amphibians. Wood frog tadpoles experimentally exposed to sediments from a highly contaminated site on the south shore experienced increased mortality and behavioural abnormalities (Savage et al. 2002). Exposure to sediments from the north shore produced cytotoxic effects on cell lines of northern leopard frogs (*Rana pipiens*) and green frogs (*R. clamitans*) (Gillan et al. 1998).

A workshop held in 1997 called 'Strategies for Assessing the Implications of Malformed Frogs' recommended the use of field investigations to verify reports of increased rates of malformations in amphibian populations with an enhanced focus on water quality (Burkhart et al. 2000). Most studies on amphibian toxicology have been designed to test the toxicity of single compounds, using laboratory exposures. These do not always give an accurate picture of the impact of contamination on wild populations and may hold little relevance for natural ecosystems. Few field studies have been conducted which measure biological endpoints and it is often difficult to isolate exposure effects due to fluctuations in environmental variables and sampling variation (Burkhart et al. 2000). We advocate a dual approach, monitoring existing amphibian populations in combination with semi-controlled field exposures (Harris et al. 2001). Raising amphibian larvae in cages placed in wetlands allows for an assessment of the impact of water quality on rates of survivorship, growth and deformity, while removing influences of confounding variables, such as predation, parasitism and interspecific competition (Harris and Bogart 1997; Harris et al. 2001).

This project was undertaken to determine the impacts of industrial and agricultural contamination on sediment quality, water quality and amphibian development in two wetlands on the north shore of the Ontario portion of the St. Lawrence at Cornwall AOC. The north shore was chosen because initial surveys had indicated areas of increased rates of deformity in leopard frogs (B. Hickey, pers. comm.) and because this area has not been as extensively studied for biological effects as the south shore. Caging is labour intensive, and requires daily visits to each site at critical times in the tadpole life history, especially during metamorphosis. The number of sites where caging can be conducted at one time is subject to logistic limitations and we were able to visit only 4 wetlands on an intensive basis. For this reason we selected two wetlands within the AOC where amphibian deformities had been previously detected.

Leopard frogs were raised to metamorphosis, and green frogs were raised to hatching, in cages within two AOC wetlands and two reference wetlands upstream of the AOC. Rates of hatching, survival and deformity, as well as time to metamorphosis were compared among study sites. The incidences of deformities of young-of-the-year (y-o-y) in wild populations of leopard frogs was also measured at wetland sites. We predicted that the rates of survival, hatching and growth would be inhibited, while the rates of deformity would be elevated in frogs in wetlands within the AOC, as compared to reference wetlands. This study was conducted over two separate field seasons in 1998 and 1999. A subsample of results from the 1998 field season has been published in an article describing the monitoring method (Harris et al. 2001). Data in this paper largely concern the overall results of the study.

Methods

Study Sites

Two wetlands within the Cornwall, Ontario, AOC and two reference wetlands upstream of the Cornwall AOC were chosen for water quality, sediment quality and frog health assessment in the summers of 1998 and 1999 (Fig. 1). Cooper Marsh and Gray's Creek are conservation areas within the Cornwall AOC, owned by the Raison Region Conservation Authority. These two sites were selected based on preliminary surveys showing elevated rates of deformity in wild frog populations (Harris et al. 2001). Cooper Marsh (Glengarry county 45°06'25''N, 74°30'43''W) is a wetland expanded by Ducks Unlimited through the flooding and diking of former agricultural land on the shore of the Lake St. Francis section of the St. Lawrence River. Frog cages were placed next to the weir where water from the St. Lawrence is pumped into Cooper Marsh for maintenance of the wetland's water level. Grays Creek is an 8 km-long waterway that flows into the St. Lawrence River, after passing through a light industrial zone, agricultural areas and under a four-lane highway. Gray's Creek Conservation Area (Glengarry county 45°02'01''N, 74°39'51''W) encompasses one hectare of land near the mouth of the creek. Frog cages

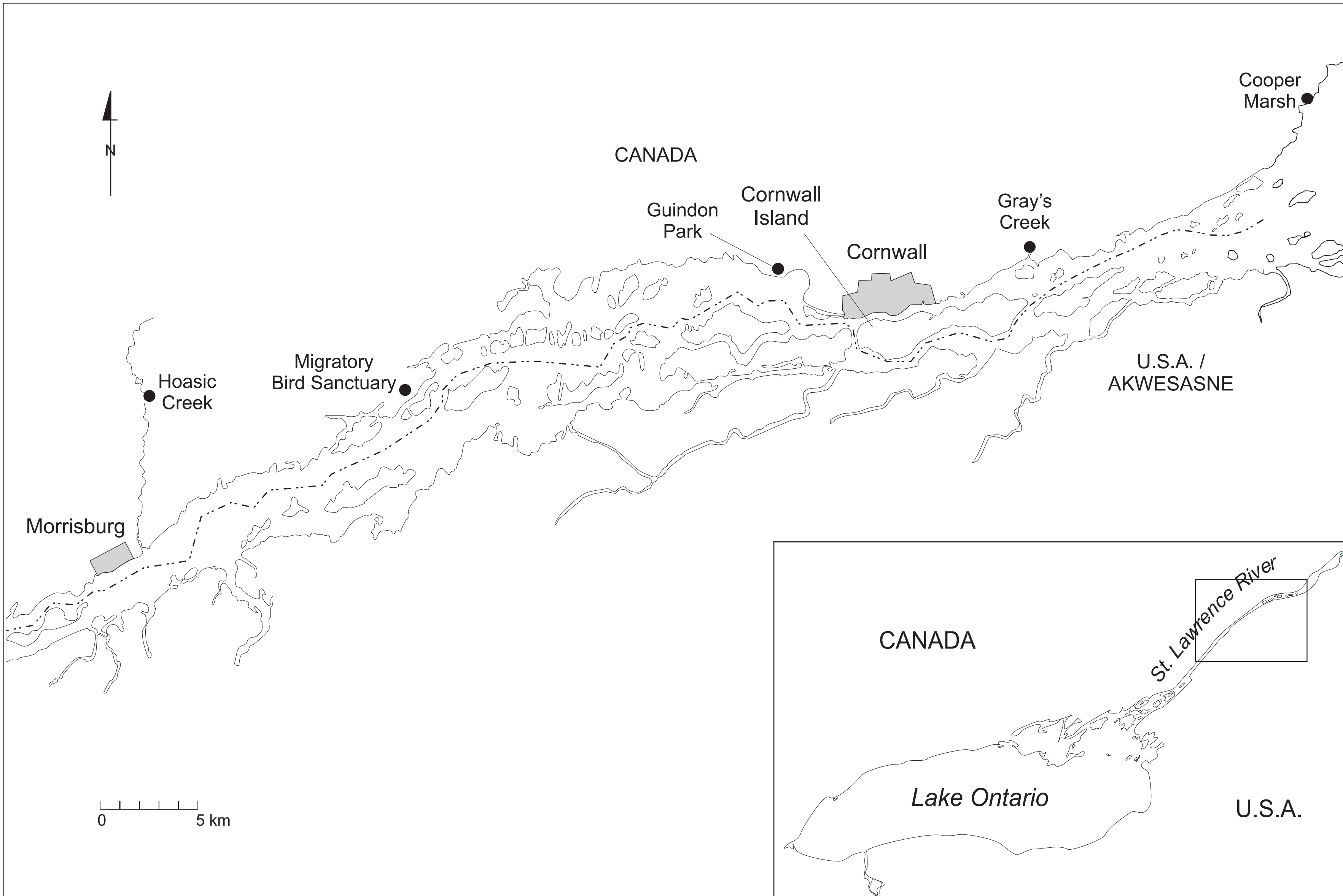

Fig. 1. Location of study sites within the St. Lawrence River at Cornwall AOC and reference sites upstream.

were placed ~0.5 km upstream from the river mouth to avoid traffic from a nearby recreational marina, and were located just downstream of a drain from a stormwater retention pond which receives stormwater runoff from the city of Cornwall.

Reference sites Hoasic Creek (sampled in 1998 and 1999) and Upper Canada Migratory Bird Sanctuary (MBS) (sampled in 1999 only), were selected based on their extended distance from industrial and agricultural areas, and because they were upstream of the Cornwall AOC (Fig. 1). At Hoasic Creek (Glengarry county 44°59'22''N, 75°11'35''W), frog cages were placed 11 km from the mouth of the creek and downstream of a large beaver marsh. MBS (Glengarry county 44°58'03''N, 75°01'21''W) is a 0.6-ha pond located beside the shore of the St. Lawrence River and west of the town of Ingleside, Ontario. The pond is used as a banding station for migratory waterfowl, and the water level is maintained throughout the year by drawing water from the St. Lawrence River. Frog cages at MBS were evenly placed around the circumference of the pond.

Leopard frog and green frog eggs used in caging studies were collected from Guindon Park Conservation Area (Glengarry county 45°01'48''N, 74°47'47''W), upstream of the Cornwall AOC and 18 km east of MBS (Fig. 1). Although it is possible that some frogs at the site could have originated in the Cornwall AOC, this site was chosen as the source of genetically robust stock, based on a preliminary survey of y-o-y leopard frogs in 1997, which found no incidences of deformities (Hickey, Unpublished data).

Water and Sediment Collection and Analysis

Water samples were taken throughout the period of ongoing frog studies: 8 and 27 June 1998, 13 and 26 May, and 9 June 1999. Water sampling was conducted using the methods of Bishop et al. (2000). Upon collection, water was immediately preserved for fixation of phosphorus (1 mL of a 30% solution of H_2SO_4 per 100-mL sample), trace metals (1 mL of 1:1 HNO_3 per 250-mL sample) and mercury (1 mL of H_2SO_4 plus 0.05% potassium dichromate per 100-mL sample). Water samples for ammonia, nitrates and nitrites were not preserved. Water samples were held at 4°C until analyses were performed.

Water samples were analyzed by Environment Canada's National Laboratory for Environmental Testing (NLET), Burlington, Ontario. The concentration of chlorides, major ions (Mg, Ca, Na and K), mercury, trace metals (Ni, Pb, An, Cr, Cu, Al), total phosphorus (TP), nitrate (NO_3-N), nitrite (NO_2-N), ammonia (NH_3-N), total Kjeldahl nitrogen (TKN), dissolved inorganic carbon (DIC) and dissolved organic carbon (DOC), as well as specific conductivity, turbidity and pH were assayed using standard methods (Environment Canada 1997b,d,e; Bishop et al. 2000). Detection limits for nutrients and major ions are listed in Table 1.

Surficial sediment was sampled from all sites on 16 June 1999. Sediment was collected using a mini-Ponar grab sampler. At least five grab samples were collected per site, approximately 1 m from each frog cage. All five or more sediment grabs from a site were pooled in a 20-L plastic bucket, thoroughly mixed and 500-mL subsamples were put into 500-mL Teflon jars for chemical analysis. Sediments were held on ice for the duration of the collection time, and then stored at –20°C within 6 h.

Particle size analysis was performed as suggested by Duncan and LaHaie (1979). Nutrients and metals (As, Se, Al, Cd, Cr, Cu, Ni, Fe, Pb, Zn and Hg) in sediments were measured using standard methods at NLET (Environment Canada 1997a,c,d; Bishop et al. 2000). PAHs in sediments were measured by gas chromatography/mass spectroscopy according to standard Environment Canada protocols (Environment Canada 1997g). The individual PAHs quantified with this method were indene, 1,2,3,4-tetrahydronapthalene, napthalene, 1- and 2-methylnapthalene, 2-chloronapthalene, acenapthylene, acenapthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(e)pyrene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, benzo(a)pyrene-D12 and benzo(g,h,i)perylene. Oil and grease were measured by subjecting a sample of sediment to acidification, then desiccating the sample and extracting oil and grease residues using hexane on a Soxhlet apparatus. The extract was collected, filtered, dried and weighed. The method detection limit was 0.5 mg/g dry weight.

Organochlorine (OC) pesticides and PCBs in sediments were analyzed through organic extraction and gas chromatography following standard methods (Environment Canada 1997f). The OCs quantified with this method were 1,3-, 1,4- and 1,2-dichlorobenzene, 1,3,5-, 1,2,4- and 1,2,3-trichlorobenzene, 1,2,3,4-tetrachlorobenzene, pentachlorobenzene, *alpha*- and *gamma*-hexachlorocyclohexane (HCH), aldrin, heptachlor epoxide, *alpha*- and *beta*-endosulfan, *alpha*- and *gamma*-chlordane, dieldrin, endrin, *p,p'*-dichlorodiphenyldichloroethylene (DDE), *p,p'*-dichlorodiphenyldichloroethane(DDD), *p,p'*-dichlorodiphenyltrichloroethane (DDT), *o,p'*-DDT, methoxychlor and mirex. Total PCBs were measured in the sediment (Environment Canada 1997f). OC pesticides were not detected in any samples at minimum detection limits ranging from 5 to 10 ng/g dry weight. Concentrations of nutrients and contaminants in sediment are given as dry weight values.

Frog Caging Studies

Caging studies were completed using early life stages of leopard frogs and green frogs; they followed one of two distinct protocols established either for embryos and tad-

TABLE 1. Means and maximums (max) of nutrients and physical characteristics of water sampled from two wetlands in the Cornwall Area of Concern (AOC) and three reference sites (1998, 1999) upstream of the AOC[a]

Site	*Compound*	*Cl (mg/L)*	*Ca (mg/L)*	*Mg (mg/L)*	*Na (mg/L)*	*K (mg/L)*	*DOC (mg/L)*[c]	*DIC (mg/L)*[d]	NO_3 NO_2 *(mg/L)*	NH_3 *(mg/L)*	*pH*	*Sp. conduct (μS/cm)*	*TKN (mg/L)*[e]	*TP (mg/L)*[f]	*Turbidity (NTU)*[g]
	MDL	0.06	0.01	0.002	0.03	0.06		0.1				0.1			0.05
	CWQG	NA[b]	NA	NA	NA	NA	NA	NA	NA	1.37–2.2	6.5–9.0	NA	NA	NA	>8 NTU change
Cooper Marsh	1998	14.9	18.9	4.14	9.64	0.26			0.25	0.11	6.85	170	0.97	0.07	1.40
	1999 mean	21.5	28.3	6.85	12.8	1.29	8.40	21.7	0.01	0.22	7.49	259	0.84	0.07	3.30
AOC	1999 max		38.5	9.62	15.2	1.52	11.1	29.9	0.02	0.24	7.68	344	0.92	0.09	7.30
Gray's Creek	1998	70.1	55.3	10.9	51.8	3.03			0.42	0.12	7.48	590	0.97	0.12	15.0
	1999 mean	82.7	73.2	19.1	75.4	2.55	11.6	44.6	0.04	0.18	7.90	863	0.86	0.09	26.0
AOC	1999 max		78.9	22.2	94.6	3.22	13.2	47.7	0.10	0.24	7.96	1030	0.99	0.12	27.0
Hoasic Creek	1998	8.10	49.1	3.22	4.46	0.06			0.03	0.03	7.05	254	1.24	0.07	0.64
	1999 mean	9.30	48.3	3.43	5.24	0.20	23.2	30.1	0.01	0.05	7.74	263	1.05	0.03	0.81
REF	1999 max		54.3	3.64	5.83	0.35	26.5	32.5	0.01	0.08	7.89	280	1.22	0.04	1.20
MBS	1999 mean	4.10	20.4	4.64	2.26	0.42	6.27	16.0	0.01	0.16	7.75	640	0.82	0.05	3.53
REF	1999 max		23.5	5.09	2.56	0.63	7.10	17.6	0.03	0.22	7.84	1600	1.09	0.06	6.4
Guindon Park **REF**	1998	1.60	39.0	9.58	1.32	1.57			0.05	0.04	7.66	254	0.61	0.03	2.1

[a]Detection limits (MDL) are listed for each compound as are Canadian Water Quality Guideline (CWQG) levels, if applicable.
[b]NA; Not applicable.
[c]DOC; Dissolved organic carbon.
[d]DIC; Dissolved inorganic carbon.
[e]TKN; Total Kjeldahl nitrogen.
[f]TP; Total phosphorus.
[g]NTU; Nephelometric turbidity unit.

poles during the first 2 to 3 weeks of development (Harris and Bogart 1997), or for tadpoles up until metamorphosis (Harris et al. 2001). Slight modifications to the established methods were necessary. Details of the 1998 caging methodology for leopard frogs were described in Harris et al. (2001). In 1999, five leopard frog egg masses (A, B, C, D and E) and in 1998, two green frog egg masses (a and b), all less than 12 h old, were collected from Guindon Park. Each egg mass represented one replicate. Egg masses were transported in coolers to the laboratory, where they were staged according to Gosner (1960); the stages of development at that time ranged from stage 6 (16-cell) to stage 14 (neural fold). Eggs were subdivided into equal portions, held overnight at ambient temperature, then transported in a cooler to the study sites. For leopard frogs, five 3-L cylindrical mesh cages were placed at each study site, and 200 eggs from one of the original five egg masses were placed in each cage. For green frogs, two cages were placed at each study site, and ~1000 (egg mass a) or 600 (egg mass b) eggs were placed in each. The 3-L cage design was described in depth in Harris and Bogart (1997). Briefly, cages were constructed from 500-mm Nytex nylon (Tekto Inc., New York), and within the cages, eggs were suspended near the water surface in a small plastic sieve.

Eggs were monitored daily until hatching. Each day, dead embryos were counted and removed with a disposable pipette. Upon hatching, any remaining dead eggs were counted and the sieve was removed to allow tadpoles to swim freely in the cage. Tadpoles were monitored by eye until they reached the active swimming and foraging stage, Gosner 23. From this point onward, boiled lettuce was given *ad libitum*, tadpoles were counted and feces removed every 2 to 3 days. During each cage check, four to five tadpoles from each cage were staged to monitor the rate of development. When tadpoles reached Gosner 25, they were transferred to the laboratory for assessment of deformities. Tadpoles were euthanized using a 2 g/L solution of tricaine methanesulfonate (MS-222) in accordance with standards of the Canada Council for Animal Care and examined under a dissecting microscope. Deformities were characterized in accordance with the Atlas of Abnormalities (Bantle et al. 1998) for *Xenopus* tadpoles.

At the time of removal for deformity assessments, 50 leopard frog tadpoles from each cage were retained at the study sites (n = 250 tadpoles per site). They were transferred to five larger 45-L cages, whose design was described in detail in Harris et al. (2001). Briefly, large cages were constructed from the same Nytex mesh, suspended within a floating wooden frame, but were further protected by an outer barrier of green garden fencing that effectively restricted juvenile muskrat (*Ondatra zibethica*) damage. Once every 1 (Gray's Creek) or 2 (all other sites) weeks, the insides of the large cages were thoroughly cleaned of algae by scrubbing with a plastic vegetable brush and rinsed several times in the ambient water. Cage maintenance at Gray's Creek was more frequent because of the high turbidity and rapid algal growth at that site. A maximum/minimum thermometer (Fisher Scientific, Canada) was placed on each cage and maximum and minimum temperatures were noted when cages were monitored.

Tadpoles were housed at densities of 0.5 per litre; 2 per litre is the recommended maximum density to avoid crowding effects in leopard frogs (Gromko et al. 1973). Also, cages were designed to minimize density effects, in that the mesh size allowed continuous water flow, thus preventing the aqueous accumulation of the growth- and development-inhibiting compounds normally secreted by tadpoles (West 1960). Feces were removed once every 2 days, thereby preventing the growth-inhibiting effects associated with fecal consumption by neighbouring tadpoles (Griffiths et al. 1993). Food was provided *ad libitum,* as studies on leopard frogs have shown this can help to eliminate density effects (Gromko et al. 1973).

Tadpoles were housed in the enclosures until at least one forelimb erupted, (Gosner 42), at which time they were removed and allowed to complete metamorphosis (Gosner 46) in the laboratory in plastic containers, slanted on a 20% grade to prevent drowning. At test termination frogs were weighed (g), then euthanized in a 2-g/L solution of MS-222. Individuals were toe-tagged with ID numbers, examined for deformities and snout-vent length was measured with vernier callipers (±0.01 mm).

Deformities were classified in accordance with the classification system for developmental abnormalities in amphibians used by Ouellet (2000). Hind limb deformities were characterized in accordance with the classification system developed by Meteyer et al. (2000) for leopard frogs. Suspected abnormalities were confirmed by radiography to detect the presence or absence of bony structures (Meteyer et al. 2000).

Surveys of Wild Leopard Frog Populations in Wetlands

We conducted surveys of wild frog populations at all sites (including Guindon Park) during June to August, 1998 and 1999. Surveys were carried out by walking a transect and capturing frogs approximately 2 m on either side of the transect with a dip net. Transects followed the shoreline (Hoasic, MBS, Gray's Creek, Guindon Park) or dikes (Cooper Marsh) and were concentrated near areas where cages were located. All captured frogs were examined for deformities on site. Weight was taken with an appropriately sized Pesola spring scale (10 ± 0.5 g for y-o-y, 50 ± 0.5 g for adults), and snout-vent length was measured with vernier callipers (±0.01 mm). Since several survey events were required for an appropriate sample size, a toe was clipped from each frog to identify any subsequent recaptures. Surveys were conducted once or twice per week at each site from 6 to 23 July 1998, and 8 June to 22 August 1999. Voucher specimens of deformed wild frogs were collected and euthanized for deformity characterization. These frogs were brought to the University of Guelph for examination by a veterinary pathologist and subjected to radiography to identify skeletal abnormalities.

Statistical Analyses

All statistical analyses were performed using STATISTICA, version 5.5 (StatSoft Inc. 2000). Sites were ranked according to concentrations of metals and nutrients in both water and sediments using a Friedman's rank test.

Rates of survivorship were evaluated at the following three developmental stages: hatching (Gosner 20), free swimming (Gosner 25) and metamorphic climax (Gosner 42). Rates of survival and deformity were compared among sites and among clutches using an ANOVA (Sokal and Rohlf 1999).

In 1999, the weight and snout-vent length of caged northern leopard frogs were compared among sites and among clutches using a two-factor (site, clutch) ANOVA. In 1998, since caged frogs were not divided into clutches, weight and snout-vent length were compared between sites using a nested ANOVA with cages nested within site (Sokal and Rohlf 1999).

Development in anuran embryos is temperature-dependent; embryos raised at higher temperatures develop at a faster rate (Bachmann 1969). Since temperature differed among sites, and to a lesser degree within sites, comparisons of time to metamorphosis in 1999 were adjusted for temperature. Degree days were calculated by taking the average of the minimum and maximum temperatures at each cage, and summing over time. The cumulative number of degree days when 50% of the tadpoles in each cage reached Gosner 42 were then compared among sites using a Kruskal-Wallis nonparametric ANOVA. Tadpoles in 1998 were not divided into clutches, and there was an uneven number of replicates per site; therefore, time to metamorphosis was analyzed using a nested ANOVA with cages nested within site using a type-3 sums-of-squares.

Rates of deformity in wild frogs were compared among sites using goodness of fit tests. Deformity rates in wild frogs were compared to a 2% background rate expected in wild populations (Ouellet 2000) and to a 5% rate considered to be potential cause for concern (Ouellet 2000) using a binomial test.

Results

Water Chemistry

Levels of TKN, which measures ammonia and organic nitrogen, exceeded 1 mg L^{-1} at Hoasic Creek (Table 1). Levels of ammonia did not exceed provincial or Canadian Water Quality Guidelines (CWQG) (Canadian Council of Ministers of the Environment 1999). The pH of water in all wetlands did not vary far from neutral (Table 1). From the cation and anion (Ca, Mg, Na, K and Cl) concentrations, we estimated that water hardness was in the medium-hard range (60–120 mg/L). Suspended solids were high at Gray's Creek, as indicated by turbidity levels of up to 27 NTU. Water from Gray's Creek had the overall highest measured concentrations of nutrients and cations; nutrient concentrations ranked significantly higher than Hoasic Creek ($q = 5.84$, $p < 0.005$) but not different than Cooper Marsh ($q = 2.8$, $p > 0.2$).

The average aqueous concentrations of two to four trace metals exceeded CWQG values for the protection of aquatic life at MBS, Cooper Marsh and Gray's Creek (Table 2). CWQG threshold values for copper, nickel, cadmium, lead and zinc were based on water hardness between 60 and 120 mg/L. Copper levels in water from MBS and Gray's Creek were slightly above the CWQG. Cadmium and chromium concentrations in Gray's Creek water were also just above CWQG values (Table 2), assuming that hexavalent chromium accounted for 10 to 60% of the total chromium in the water. Aluminium concentrations exceeded the CWQG value of 0.1 mg/L in water samples from all sites except Hoasic Creek (Table 2). Gray's Creek ranked highest for trace metals in the water, followed by MBS, Cooper Marsh, and Hoasic Creek but differences were not significant.

Sediment Chemistry

The physical characteristics of the sediments varied among sites. Sediments at Cooper Marsh and MBS were dominated by clay and silt, while those at Hoasic Creek and Gray's Creek contained higher proportions of sand and gravel (Table 3). There was a significant correlation between concentrations of trace metals in the water and those in the sediment ($r = 0.63$, $p < 0.05$). Oil and grease were detected in sediment from all sites (Table 3), but concentrations were below PSQG standard values. The site with the highest level of oil and grease was Hoasic Creek. Although a reference site, the cages were located adjacent to a road where runoff from automobile traffic could enter the creek. Nutrient concentrations in the sediments were high at all sites, as indicated by values for TKN and TP that often exceeded PSQG for low effects (Table 3); TKN values exceeded PSQG for severe effects at Hoasic Creek. Concentrations of TOC also exceeded the PSQG standard at all sites, approaching severe effect levels at Hoasic Creek.

Concentrations of chromium, copper and nickel in surficial sediments exceeded the PSQG lowest effect levels (LEL) at all sites except Hoasic Creek (Table 3). Levels of chromium in the sediments of Cooper Marsh (107 µg/g) were just under the PSQG severe effect value

TABLE 2. Concentrations of metals (mg/L) in water samples collected in 1998 (n = 2) and 1999 (n = 3) from two wetlands in the Cornwall AOC and three reference sites upstream of the AOC[a]

Site		*Year*	*Al*	*Cd*	*Cr*	*Cu*	*Ni*	*Pb*	*Zn*
CWQG			*0.1*[b]	*0.00017*[c]	*0.0001*[d]	*0.002*[c]	*0.065*[c]	*0.002*[c]	*0.03*
Cooper Marsh	**AOC**	1998 mean	0.024	0.0001	0.0002	0.0002	0.0002	0.0002	0.0010
		1998 max	0.024	0.0001	0.0002	0.0002	0.0002	0.0002	0.0010
		1999 mean	**0.148**	0.0001	0.0005	0.0006	0.0004	0.0004	0.0010
		1999 max	**0.391**	0.0001	**0.0012**	0.0011	0.0008	0.0007	0.0013
Gray's Creek	**AOC**	1998 mean	**1.118**	**0.0002**	**0.0023**	**0.0031**	0.0020	0.0012	0.0117
		1998 max	**1.320**	**0.0002**	**0.0024**	**0.0044**	0.0022	0.0018	0.0164
		1999 mean	**1.222**	**0.0002**	**0.0019**	**0.0024**	0.0019	0.0012	0.0088
		1999 max	**1.350**	**0.0002**	**0.0021**	**0.0026**	0.0021	0.0020	0.0092
Hoasic Creek	**REF**	1998 mean	0.009	0.0001	0.0002	0.0003	0.0003	0.0002	0.0031
		1998 max	0.009	0.0001	0.0002	0.0003	0.0003	0.0002	0.0037
		1999 mean	0.041	0.0001	0.0002	0.0003	0.0003	0.0002	0.0005
		1999 max	0.099	0.0001	0.0003	0.0006	0.0005	0.0002	0.0009
MBS	**REF**	1999 mean	**0.225**	0.0001	0.0004	0.0016	0.0006	0.0004	0.0016
		1999 max	**0.470**	0.0001	0.0007	**0.0037**	0.0008	0.0008	0.0033
Guindon Park	**REF**	1998	**0.208**	0.0001	0.0003	0.0012	0.0007	0.0002	0.0018

[a]Bold numbers exceed the Canadian Water Quality Guidelines (CWQG) for the protection of aquatic life.
[b]Related to pH, Ca, DOC.
[c]Water hardness 60–120 mg/L.
[d]Hexavalent chromium.

(110 µg/g). Gray's Creek sediments also exceeded the PSQG value for zinc. Concentrations of aluminium ranged from 8410 to 45,100 µg/g; there are no provincial or federal guidelines for comparison. Cadmium, lead, mercury and arsenic were detected in all sediment samples, but were well below PSQG levels. Overall, Cooper Marsh ranked highest for heavy metal concentrations, followed by MBS, Gray's Creek and Hoasic Creek. The difference between Cooper Marsh and Hoasic Creek samples was significant ($q = 3.74$, $p < 0.05$).

Levels of OC pesticides were below detection limits for all compounds at all sites. PCBs were found in sediments at all sites, while PAHs were only found at MBS and Gray's Creek (Table 3). All values were below PSQG effect levels.

Frog Caging Studies

Green frogs. Rates of deformity in green frog tadpoles at 11 days of age (Gosner 25) ranged from 4 to 5% at Cooper Marsh and Hoasic Creek, and up to 20.5% at Gray's Creek. A log-linear analysis indicated a significant interaction between egg mass and site, thus deformities were analyzed separately for each egg mass. For Clutch B, rates of deformity differed significantly among sites ($\chi^2 = 34.89$, $p < 0.001$); tadpoles were more likely to be deformed at Gray's Creek than at Hoasic Creek or Cooper Marsh.

Leopard frogs—survivorship. Survivorship at all three life stages censused—hatching, Gosner 25 and Gosner 42—was uniformly high. Some significant differences were found among sites; however, survivorship never fell below 75% at any site. The hatching success of leopard frog eggs was high at all sites, not falling below a mean of 97%; there was no difference in hatching success among sites or among egg masses. Tadpole survival to Gosner 25 was also relatively high at all sites: Cooper Marsh 86%, MBS 87.6%, Gray's Creek 88.2% and Hoasic Creek 91%. Nonetheless, there was a significant interaction between site and survivorship ($\chi^2 = 23.7$, $p < 0.001$), with tadpole survival being higher at Hoasic Creek than at any other site. Survival to metamorphosis (Gosner 42) was also high at all sites: MBS 88.4%, Hoasic Creek 89.2%, Cooper Marsh 90% and Gray's Creek 93.2%. A log-linear analysis indicated a significant three-way interaction among site, clutch and survivorship to metamorphosis ($\chi^2 = 25.8$, $p = 0.01$); thus, clutches were analyzed individually. Only clutch B showed a significant interaction between site and survival to metamorphosis, with survivorship being lower at all sites compared to at MBS.

Leopard frogs—deformities. Rates of deformity in caged tadpoles were low, but there were still significant differences among sites. Average deformity rates in Gosner 25 tadpoles ranged from 0.2% at MBS to 2% at Gray's Creek. The most common type of deformity was

TABLE 3. Composition and concentrations of metals, nutrients, PAHs, total PCBs and oil and grease in surficial sediments collected from study sites in 1999[a]

		Reference Wetlands		AOC Wetlands	
Compound	*PSQG*	*MBS*	*Hoasic Creek*	*Gray's Creek*	*Cooper Marsh*
Metals					
Aluminum	no LEL	32,100	8410	26,200	45,100
Arsenic	6	2	1	3	3
Cadmium	0.6	0.4	0.6	0.2	0.4
Chromium	26	**49**	23	**40**	**107**
Copper	16	**25.5**	6.3	**21.1**	**52.0**
Lead	31	14.9	10.8	12.9	13.9
Mercury	0.2	0.05	0.09	0.04	0.04
Nickel	16	**25.2**	4.2	**22.8**	**54.5**
Zinc	120	98	75	**142**	115
Nutrients					
TKN[c]	550	**2830**	**5230**[g]	**1090**	**3830**
TP[d]	600	**756**	552	**648**	581
NO_2	no LEL	0.36	0.53	0.58	0.26
NO_3	no LEL	0.04	<ND	ND	0.2
%TOC[e]	1	**4.06**	**8.92**	**1.65**	**7.05**
PAHs[h]					
Fluoranthene	0.75	<0.053	<0.053	0.0922	<0.053
Chrysene	0.34	<0.05	<0.05	0.0621	<0.05
Perylene	NA[b]	0.119	<0.058	0.107	<0.058
Total PAHs	4	0.119	<0.05	0.261	<0.05
Oil and Grease mg/g	1.5	0.61	1.03	0.70	0.73
Total PCBs ng/g	70	34.4	60.3	45.1	23.3
Composition					
% Gravel		0	9.1	2.61	0
% Sand		11.08	54.96	50.47	0.18
% Silt		60.36	20.05	28.45	28.26
% Clay		28.56	15.9	16.79	71.56
% LOI[f]		10.23	18.05	6.77	16.64
% Moisture		64.65	100	53.66	86.1

[a]Bold numbers exceed the lowest effect levels (LEL) of the Ontario Provincial Sediment Quality Guideline (PSQG: Persaud et al. 1992). All values are expressed in µg/g dry weight unless otherwise stated.
[b]NA; Not applicable.
[c]TKN; Total Kjeldahl nitrogen.
[d]TP; Total phosphorus.
[e]TOC; Total organic carbon.
[f]LOI; Loss of weight on ignition.
[g]Exceeded severe effect level of 4800 µg/g.
[h]Out of 25 PAHs tested, 22 were not detected at any of the study sites.

dorsal or lateral flexure of the notochord. The log-linear model, site(S) x deformities(D) clutch(C) x site(S), which excludes the interaction between clutch and deformity rate, gave the best fit of the data (χ^2 = 11.85, p = 0.75) and indicated that there was no clutch effect on the rate of deformities. For this reason, tadpoles from different clutches were pooled and frequencies of deformity among sites compared directly. Rates were significantly different among sites (χ^2 = 11.5, p = 0.009), with tadpoles from Cooper Marsh and Gray's Creek being more likely to be deformed than those from Hoasic Creek and MBS.

Deformity rates in newly metamorphosed frogs did not differ among sites (Table 4). Types of deformities included missing eyes (anophthalmy), extra toes (polyphalangy), missing toes (ectrodactyly), lateral flexure of the spinal chord and edema. Several metamorphs had a bubble of fluid within the toe tip, which was caused by infections of parasitic copepods; these were not counted as deformities. In 1999, average deformity rates among sites varied from 0.9% at Gray's Creek to 3.4% at Cooper Marsh. In 1998, average rates varied from 0% at Hoasic Creek to 2.2% at Gray's Creek. A log-linear analysis revealed no relationship between site and deformity rate (χ^2 = 8.1, p = 0.92) or clutch and deformity rate (χ^2 = 7.4, p = 0.96).

Leopard frogs—size at and time to metamorphosis. Overall, there was no significant difference in the mean

TABLE 4. Rates of deformity in caged leopard frog tadpoles at Gosner 25 in 1999 and Gosner 46 in 1998 and 1999

		Deformities %		
		Stage 25	*Stage 46*	
Site		*1999*	*1998*[b]	*1999*
MBS	mean	0.2[a]		2.6
REF	std dev	0.4		1.8
	n	740		212
Hoasic Creek	mean	0.4[a]	0	1.2
REF	std dev	0.6	0	1.8
	n	711	108	224
Gray's Creek	mean	2.0	2.2	0.9
AOC	std dev	2.2	2.6	2.0
	n	508	186	223
Cooper Marsh	mean	1.6	1.3	3.4
AOC	std dev	1.3	1.0	1.3
	n	391	228	225

[a]Indicates significance at the $p < 0.05$ level.
[b]Data for 1998 are from Harris et al. (2001).

mass of newly metamorphosed leopard frogs in cages among study sites in either 1998 or 1999 (data not shown); however, there was a significant clutch effect and a significant interaction between clutch and site in 1999. There were significant differences in the mean masses of frogs originating from different clutches ($F = 11.63$, $p < 0.01$). Frogs from egg masses B and E were significantly larger than those from masses A, C and D. In 1999, there was a significant difference in the mean snout-vent length of caged frogs among sites ($F = 3.1$, $p = 0.025$), but the difference accounted for less than 3% of body length. Frogs at Cooper Marsh (mean = 21.37 mm) were significantly larger than frogs from Hoasic Creek (mean = 20.72 mm, $p < 0.03$).

In 1998, we were unable to detect a significant difference in time to metamorphosis among sites, after excluding cages attacked by muskrats (nested ANOVA, $F = 1.078$, $p = 0.4$); however, in 1999, with a more complete data set, tadpoles appeared to reach Gosner 42 sooner at AOC sites than at reference sites. Development in amphibians is temperature-dependent and temperature did vary significantly among sites (Kruskal-Wallis ANOVA, $H = 11.96$, $p < 0.0075$); therefore we corrected for differences in temperature by comparing the number of cumulative degree days when 50% of tadpoles in each cage reached Gosner 42. The number of cumulative degree days was highest at reference sites and lowest at sites within the AOC (Kruskal-Wallis ANOVA, $F = 15.3$, $p = 0.002$, Fig. 2).

The average number of days between the first and last tadpole in each cage achieving Gosner 42 also varied significantly among sites (two factor ANOVA, $F = 54.1$, $p < 0.001$), with metamorphosis taking place faster in the AOC (means of 3.5 and 6.5 days) versus reference sites (means of 9.5 and 11.5 days) (Fig. 3).

Surveys of Wild Leopard Frog Populations in Wetlands

Deformity rates of wild leopard frogs ranged from 4.7 and 10% at Gray's Creek in 1999 and 1998, respectively, to 0% at Hoasic Creek (Table 5). The majority of cases involved hind limb deformities, mainly polyphalangy or ectrodactyly. Also found were asymmetrical eyes, severely malformed hind and forelimbs, missing appendages and skin growths. Deformity rates varied between years and qualitatively. In 1998, deformity rates in y-o-y frogs were statistically greater than a 2% background rate at sites within the AOC, but not at reference sites (Table 5). In 1999, the same trend occurred; but rates above 2% were only seen when considering frogs of all ages. Deformity rates were not significantly greater than 5% in any of the sites, in either year. Most deformities were found in y-o-y in 1998, but most were found in frogs at least one year old in 1999 suggesting that these may be frogs that emerged in the 1998 field season. In order to assemble a reasonable sample size for statistical comparison of deformity rates among sites, data from wild frogs collected in 1998 ($n = 279$) and 1999 ($n = 612$) were pooled. Hoasic Creek was excluded from the analysis due to low sample size ($n = 37$). Overall, there was no difference in the deformity rates of y-o-y frogs among sites ($\chi^2 = 2.75$, $p = 0.25$).

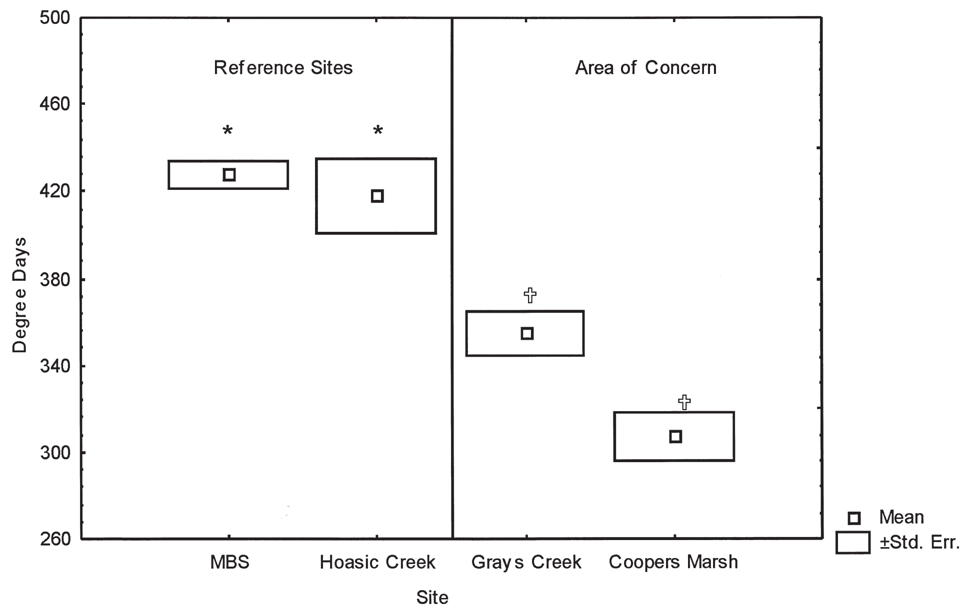

Fig. 2. The total number of cumulative degree days at which 50% of the tadpoles had reached Gosner 42 (metamorphic climax). Northern leopard frog tadpoles raised at sites within the Cornwall AOC were exposed to significantly fewer degree days by the onset of metamorphosis compared to tadpoles raised at reference sites. Sites that were significantly different from one another are denoted by * and ☦.

Discussion

All of our study sites were relatively eutrophic, as indicated by elevated levels of TP and TKN in the water. Average levels of TKN ranged from 0.61 to 1.24 mg/L, and levels over 0.6 mg/L are considered to be within the eutrophic range for surface waters. Levels of nitrates/nitrites and ammonium were not elevated and were well below levels known to have sublethal effects on amphibian larvae (Rouse et al. 1999).

Levels of trace metals in water from three of our four study sites exceeded CWQG values for the protection of aquatic life in at least one of the two years of study. Aqueous copper concentrations in Gray's Creek and Hoasic Creek, chromium concentrations in Cooper Marsh and Gray's Creek, and aluminium concentrations in all site waters except Hoasic Creek were above recommended maximum values. At Gray's Creek, which ranked highest for aqueous trace metal concentrations, aluminium concentrations were an order of magnitude higher than CWQGs and exceeded levels which caused mortality in early stage (Gosner 25) leopard frog tadpoles (Freda and McDonald 1990).

Metals in sediments of the St. Lawrence River measured by Filion and Morin (2000) may not accurately reflect the exposure of biota in AOC wetlands. They analyzed metal content in the shallow littoral zone sediments of the river in the Cornwall AOC. Metal concentrations

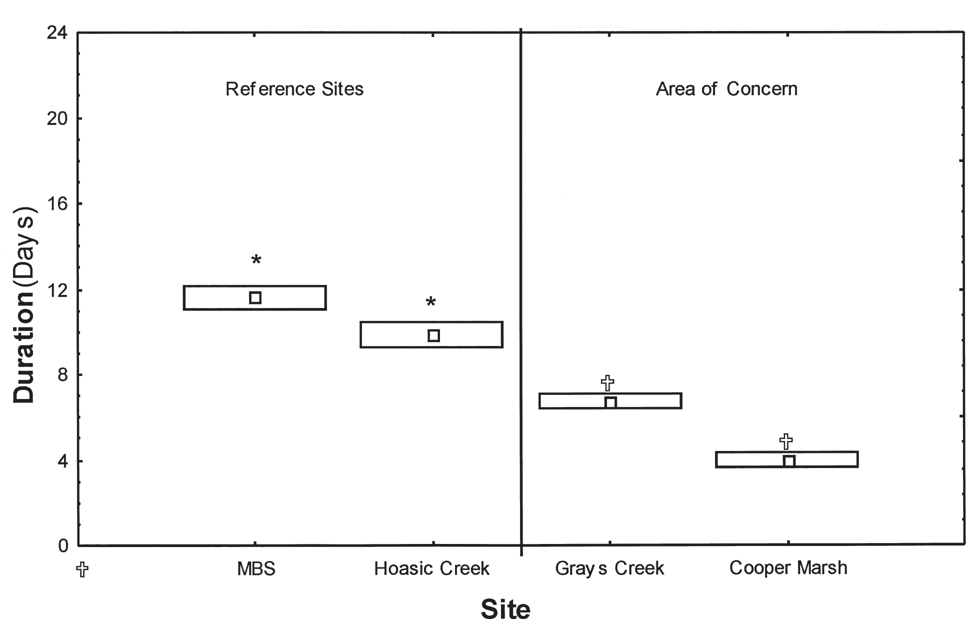

Fig. 3. Tadpoles from sites within the AOC metamorphosed more synchronously than those within reference sites as indicated by the duration of time over which metamorphosis took place. Sites that were significantly different from one another are denoted by * and ☦.

TABLE 5. Rates of deformity (%) in wild leopard frogs in 1998 and 1999[a]

		N Captured		*Deformity rate (%)*	
Site	*Year*	*Total*	*y-o-y*	*Total*	*y-o-y*
MBS **REF**	1999	127	113	0.9	0.9
Hoasic	1998[c]	16	16	0	0
Creek	1999	21	15	0	0
REF	Total	37	31	0	0
Guindon	1998	76	74	4.1	4.1
Park	1999	43	19	2.3	5.2
REF	Total	119	93	3.4	4.3
Gray's	1998	30	20	6.6	10.0[b]
Creek	1999	149	108	4.7[b]	1.9
AOC	Total	179	128	5.0	3.1
Cooper	1998	157	67	2.5	6.0[b]
Marsh	1999	272	165	4.0[b]	3.6
AOC	Total	429	232	3.5	4.3[b]

[a]Deformity rates are subdivided into young of year (y-o-y) and all ages combined.
[b]Indicates deformity rates significantly greater than a background rate of 2% (binomial test).
[c]Data for 1998 are from Harris et al. (2001).

that they measured were well below Ontario PSQGs (Persaud et al. 1992); which they attributed to the low proportion of organic matter and clay in the sandy, littoral zone sediments of the river. Wetlands, in comparison, tend to have high proportions of silt and clay in the sediments. In contrast to metals in shallow riverine sediments, concentrations of heavy metals and nutrients in our shallow wetland sediments were elevated, exceeding PSQGs, and in some cases reaching severe effect levels for biota. In particular, copper, chromium, nickel and zinc in the sediments of some or all of the study wetlands exceeded Ontario PSQGs (Persaud et al. 1992). Sediments at all four study sites were nutrient-rich, as was true of the water, and exceeded PSQGs for TKN. Residues of PAHs and organochlorines, including PCBs, were low at all sites. Sediment contaminant profiles in Cooper Marsh and Gray's Creek were typical of the north shore of the AOC, containing elevated concentrations of heavy metals, but not the elevated concentrations of PCBs and PAHs associated with industrial sources on the south shore (Dreier et al. 1997).

The hatching success and survivorship to metamorphic climax of leopard frog egg masses was high at all sites, with no significant differences among sites within the Cornwall AOC and reference sites. There were no associations between hatching success and trace metal concentrations for leopard frogs, even though aluminium levels at our sites exceeded those demonstrated to severely depress hatching success in a variety of amphibian larvae (Freda and McDonald 1990; Freda et al. 1990). Early stage survivorship in leopard frog tadpoles was slightly but significantly higher at reference sites compared to sites within the Cornwall AOC. However, it is doubtful that the differences were biologically relevant given that the survivorship of tadpoles never fell below 75%. In general, survivorship results indicated that water quality at all sites was sufficient for the successful reproduction of leopard frogs and showed no evidence of reproductive impairment.

Several chemical characteristics of the water at our sites had important implications for the toxicity of metals to aquatic organisms. The neutral to slightly alkaline range of pHs found at all sites would probably have reduced the toxicity of most metals, compared to acidic conditions. The toxicity of aluminium to anuran larvae, including leopard frogs, is highly pH-dependent with free Al ions being more prevalent at low pHs, between pH 4.0 and 5.0, and aluminium hydroxy complexes forming above pH 5.0 (Freda 1991). Aluminium can also complex with dissolved organic compounds which occur naturally in pond water. Levels of DOC above 5 mg/L, which was exceeded at all sites, can virtually eliminate aluminium toxicity (Freda et al. 1990). Divalent cations such as calcium and magnesium as well as increased levels of sodium decrease metal toxicity by competing with metal ions for binding sites on respiratory surfaces (Jackson et al. 2000). For this reason, hard water often mitigates metal toxicity in acute exposures of amphibian larvae; however, chronic exposure studies in amphibians have not demonstrated a similar effect (Freda 1991).

Elevated deformity rates in AOC wildlife are defined as a beneficial use impairment (International Joint Commission 1987). While rates of deformity in Gosner 25 caged leopard frog tadpoles were significantly higher at sites within the Cornwall AOC compared to reference sites, they were comparable to the 2% frequency predicted as a background level in frog populations (Ouellet 2000). Rates of deformity in metamorphosing caged leopard frogs were also low, varying between 1 and 2% among sites. The rates expressed in caged leopard frogs did not indicate that there was a beneficial use impairment of this species in the two wetlands we studied within the AOC. Potential stressors or teratogenic agents were not present within the caged environment in which the frogs were raised. If teratogenic chemicals in the environment were principally responsible for elevated rates of deformity seen in wild frogs, then they were not present in high enough quantities in the water of these study sites to induce deformities. It is possible that caging reduces other stressors on tadpoles that act synergistically with contaminants to produce elevated rates. Tadpoles raised in cages were protected from some nutritional, competitive and disease stressors.

We found significant differences in the onset and duration of metamorphosis of caged tadpoles. Tadpoles from sites within the Cornwall AOC metamorphosed significantly earlier and in a more synchronous fashion than did tadpoles from reference sites. Onset of metamorphosis is a phenotypically plastic trait (Wilbur and Collins 1973; Denver et al. 1998) and many natural or ecological factors are involved in its physiological induction. These factors include density, temperature, food availability and habitat quality (Newman 1998). We attempted to address these factors in our study design by keeping conditions as uniform as possible among sites. Water temperature, which could not be controlled, was not responsible for differences in the onset of metamorphosis as demonstrated by the fewer number of degree days required for metamorphosis within the Cornwall AOC.

It is possible that differences in onset of metamorphosis among sites are the result of chemical contamination. Some chemicals act as antagonists of thyroid function and inhibit or reduce the peak in thyroid hormone that normally triggers the initiation of amphibian metamorphosis (Fort et al. 2000), thus leading to a delay in metamorphosis.

Tadpoles metamorphosing earlier often do so at the cost of a reduced body size at metamorphosis and have a decreased post metamorphosis survivorship (Beck and Congdon 1999). However, caged frogs at sites where metamorphosis was accelerated did not show a significant reduction in body size. Snout-vent length was slightly, but significantly, larger at sites within the AOC as compared to reference sites, indicating that these frogs had faster growth rates.

Considering the levels of metals in the sediments from the region, and the documentation of delayed growth and development in tadpoles exposed to metals (Lefcort et al. 1998), it would be interesting to repeat caging studies and include exposure to sediments. The observed acceleration in metamorphic rates might be reversed under such an exposure regime.

Consistent differences were seen among clutches in leopard frogs in terms of survivorship at various life stages and growth. Perhaps even more important, significant interaction terms were seen between clutch and site, indicating that clutches responded differently to environmental conditions at sites. Such interclutch variability is thought to be the result of maternal effects arising from differences in egg size or maternal investment per egg (Parichy and Kaplan 1992). There is evidence to suggest that both maternal and genetic effects can lead to differences in tolerance to suboptimal water chemistry. Acid tolerance in wood frogs is highly dependent on maternal effects, and to some degree genetic effects (Pierce and Sikand 1985). This variation in tolerance to water chemistry, growth rates and time to metamorphosis due to maternal effect has important implications for both field exposure studies and lab toxicity testing. More than ever it emphasizes the importance of using multiple clutches in amphibian bioassays.

Summary and Conclusions

Water and sediment within two AOC wetlands contained elevated levels of metals and nutrients, some exceeding federal and provincial guidelines for the protection of aquatic life. These same sites had significantly higher rates of malformations in caged leopard frog tadpoles; time to metamorphosis was reduced and metamorphosis was more rapid within the AOC, than in the reference wetlands.

While associations between contaminant levels and biological endpoints are correlative, we attempted to account for or remove as much as possible, variation due to environmental factors. Caging is a useful method to help control environmental factors in field exposures, such as density, food availability and predation, which may impact on biological endpoints.

No evidence for beneficial use impairment of leopard frogs was found in the two wetlands studied within the AOC, either in terms of reproductive impairment or deformities. However, only two wetlands within the AOC were examined. Further work, in a larger number of wetlands is required in order to definitively assess whether or not amphibian reproductive success is impaired within the AOC.

Acknowledgements

We thank the following for assistance: Sara Ashpole, Carole Mitchell, Chris Thompson, Barb Martinovic and Glenn Barrett. We would also like to thank the Raisin Region Conservation Authority for allowing us access to sites and the St. Lawrence River Institute of Environmental Sciences for providing us with laboratory space. We are grateful for the scientific input of Brian Hickey, James P. Bogart, Phillip Byrnes and Doug Campbell and to D.V. Weseloh for reading earlier editions of this manuscript.

References

Bachmann K. 1969. Temperature adaptations of amphibian embryos. Amer. Natur. **103:**115–130.

Bantle JA, Dumont JN, Finch RA, Linder G, Fort DJ. 1998. Atlas of abnormalities: a guide for the performance of FETAX. Second Ed. Oklahoma State University Press, Oklahoma.

Beck CW, Congdon JD. 1999. Effects of individual variation in ages and size at metamorphosis on growth and survivorship of southern toad (*Bufo terrestris*) metamorphs. Can. J. Zool. **77:**944–951.

Bishop CB, Gendron AD. 1998. Reptiles and amphibians: shy and sensitive vertebrates of the Great Lakes basin and St. Lawrence River. Environ. Monit. Assess. **53:**225–244.

Bishop C, Struger J, Shirose L, Dunn L, Campbell D. 2000. Contamination and wildlife communities in storm water detention ponds in Guelph and the Greater Toronto Area, Ontario (1997, 1998) Part II. Water Qual. Res. J. Canada **35:**437–474.

Burkhart JG, Ankley J, Bell H, Carpenter H, Fort DJ, Gardner D, Gardner H, Hale R, Helgen JC, Jepson P, Johnson D, Lannoo M, Lee D, Lary J, Levey R, Magner L, Meteyer C, Shelby MD, Lucier G. 2000. Strategies for assessing the implications of malformed frogs for environmental health. Environ. Health Perspect. **108:**83–90.

Canadian Council of Ministers of the Environment (CCME). 1999. Canadian water quality guidelines for the protection of aquatic life. Canadian Council of the Ministers of the Environment, Winnipeg.

Calevro F, Campani S, Ragghianti M, Bucci S, Mancino G. 1998. Tests of toxicity and teratogenicity in biphasic vertebrates treated with heavy metals (Cr^{3+}, Al^{3+} and Cd^{2+}). Chemosphere **37:**3011–3017.

de Solla SR, Bishop CA, Lickers H, Jock K. 2001. Organochlorine pesticides, PCBs, dibenzodioxin, and furan concentrations in common snapping turtle eggs (*Chelydra serpentina serpentina)* in Akwesasne, Mohawk territory, Ontario, Canada. Arch. Environ. Contam. Toxicol. **40:**410–417.

Denver RJ, Mirhadi N, Phillips M. 1998. Adaptive plasticity in amphibian metamorphosis: response of *Scaphiopus hammondii* tadpoles to habitat desiccation. Ecology **79:**1859–1872.

Dreier SI, Anderson J, Biberhofer J, Eckersley M, Helliar R, Hickey M, Richman L, Stride F. 1997. Remedial action plan for St. Lawrence River (Cornwall) area of concern stage 2 report: the recommended plan. Environment Canada.

Duncan GA, LaHaie GG. 1979. Size analysis procedures used in the sedimentology laboratory. Hydraulics Division, NWRI, CCIW, Environment Canada, Burlington, Ontario.

Environment Canada. 1997a. Determination of mercury in liquid and solid samples by cold-vapour atomic absorption. Section INWI/S1. Water Technology International Corporation, Burlington, Ont.

Environment Canada. 1997b. Determination of metals (total and dissolved) in water by inductively couple plasma (ICP). Section INW14. Water Technology International Corporation, Burlington, Ont.

Environment Canada. 1997c. Determination of total and extractable metals in solids. Section INSO Water Technology International Corporation, Burlington, Ont.

Environment Canada. 1997d. Manual of analytical methods. Volume 1: major ions and nutrients. The National Laboratory for Environmental Testing, Burlington, Ont.

Environment Canada. 1997e. Manual of analytical methods. Volume 2: trace metals. National Laboratory for Environmental Testing, Burlington, Ont.

Environment Canada. 1997f. Method for the analysis of PCBs and organochlorine pesticides in water. Section GSW1. Water Technology International Corporation, Burlington, Ont.

Environment Canada. 1997g. Method for the determination of polynuclear aromatic hydrocarbons in solids by GC/MS. Section MSW2. Water Technology International Corporation, Burlington, Ont.

Filion A, Morin A. 2000. Effect of local sources on metal concentrations in littoral sediments and aquatic macroinvertebrates of the St. Lawrence River, near Cornwall Ontario. Can. J. Fish. Aquat. Sci. **57:**113–125.

Fort DJ, Rogers RL, Morgan LA, Miller MF, Clark PA, White JA, Paul RR, Stover EL. 2000. Preliminary validation of a short term morphological assay to evaluate adverse effects on amphibian metamorphosis and thyroid function using *Xenopus laevis*. J. Appl. Toxicol. **20:**419–425.

Freda J. 1991. The effects of aluminium and other metals on amphibians. Environ. Pollut. **71:**305–328.

Freda J, Cavdek V, McDonald DG. 1990. Role of organic complexion in the toxicity of aluminium to *Rana pipiens* embryos and *Bufo americanus* tadpoles. Can. J. Fish. Aquat. Sci. **47:**217–224.

Freda J, McDonald DG. 1990. Effects of aluminium on the leopard frog, *Rana pipiens*: life stage comparisons and aluminium uptake. Can J. Fish. Aquat. Sci. **47:**211–216.

Gillan KA, Hasspieler BM, Russell RW, Aadeli K, Haffner GD. 1998. Ecotoxicological studies in amphibian populations of southern Ontario. J. Great Lakes Res. **24:**45–54.

Gosner KL. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica **16:**183–190.

Griffiths RA, Denton J, Wong AL. 1993. The effect of food level on competition in tadpoles: interference mediated by protothecan algae? J. Anim. Ecol. **62:**274–279.

Gromko M, Francene H, Mason S, Smith-Gill SJ. 1973. Analysis of the crowding effect in *Rana pipiens* tadpoles. J. Exp. Zool. **186:**63–72.

Harris ML, Bishop CA, McDaniel TV. 2001. Assessment of rates of deformity in wild frog populations using in situ cages: a case study of leopard frogs (*Rana pipiens*) in Ontario, Canada. Biomarkers **6:**52–63.

Harris ML, Bogart JP. 1997. A cage for evaluation of in situ water quality using frog eggs and tadpoles. Herp. Review **28:**134–135.

Hickey MB. Unpublished data.

Internation Joint Commision. 1978. Great Lakes water quality agreement. IJC, Windsor, Ont.

International Joint Commision. 1987. Revised Great Lakes water quality agreement of 1978. IJC, Windsor, Ont.

Jackson BP, Lasier PJ, Miller WP, Winger PW. 2000. Effects of calcium, magnesium, and sodium on alleviating cadmium toxicity to *Hyalella azteca*. Bull. Environ. Contam. Toxicol. **64:**279–286.

Lefcort H, Meguire RA, Wilson LH, Ettinger WF. 1998. Heavy metals alter the survival growth, metamorphosis and anti-predatory behavior of Columbia Spotted frog (*Rana luteiventris*) tadpoles. Arch. Environ. Contam. Toxicol. **35:**447–456.

Martinovic B, Lean D, Bishop CA, Birmingham E, Secord A, Jock K. 2003. Health of tree swallow (*Tachycineta bicolor*) nestlings exposed to chlorinated hydrocarbons in the St. Lawrence River Basin. Part II. Basal and stress plasma corticosterone concentrations. J. Toxicol. Environ. Health A **66:**2015–2029.

Meteyer CU, Loeffler IK, Fallon JF, Converse KA, Green E, Helgen JC, Kersten S, Levey S, Eaton-Poole L, Burkhart JG. 2000. Hind limb malformations in free-living leopard frogs (*Rana pipiens*) from Maine, Minnesota and Vermont suggest multiple etiologies. Teratology **62:**151–171.

Newman RA. 1998. Ecological constraints on amphibian metamorphosis: interactions of temperature and larval density with responses to changing food level. Oecologia **11:**9–16.

Ouellet M. 2000. Amphibian deformities: current state of knowledge, p. 617–646. *In* Sparling DW, Bishop CA, Linder G (ed.), Ecotoxicology of amphibians and reptiles. Society of Environmental Toxicology and Chemistry, Pensacola, Fla.

Parichy DM, Kaplan RH. 1992. Maternal effects on offspring growth and development depend on environmental quality in the frog *Bombina orientalis*. Oecologia **91:**579–586.

Persaud D, Jaagumag R, Hayton A. 1992. Guidelines for the protection and management of aquatic sediment quality in Ontario. Ontario Ministry of Environment, Water Resources Branch. Toronto, Ont.

Pierce BA, Sikand N. 1985. Variation in acid tolerance in wood frogs: genetic and maternal effects. Can. J. Zool. **63:**1647–1651.

Rouse JD, Bishop CA, Struger J. 1999. Nitrogen pollution: an assessment of the impact on amphibians. Environ. Health Perspect. **107:**1–6.

Savage WK, Quimby FW, DeCaprio AP. 2002. Lethal and sublethal effects of polychlorinated biphenyls on *Rana sylvatica* tadpoles. Environ. Toxicol. Chem. **21:**168–174.

Sokal RR, Rohlf FL. 1999. Biometry. Second Ed. W.H. Freeman and Co., New York.

StatSoft, Inc. 2000. STATISTICA for Windows (Computer program manual). Tulsa, Okla.

West LB. 1960. The nature of growth inhibitory material from crowded *Rana pipiens* tadpoles. Physiol. Zool. **33:**232–239.

Wilbur HM, Collins JP. 1973. Ecological aspects of amphibian metamorphosis. Science **182:**1305–1314.

Received: May 30, 2003; accepted: June 1, 2004.